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(54) Methods for generating polynucleotides having desired characteristics by iterative selective and recombination

(57) The present invention relates to a method for evolving a polynucleotide encoding a plurality of genes, e.g. multiple genes forming a multicomponent pathway. The method involves shuffling of polynucleotides by conducting a polynucleotide amplification process on overlapping segments of a population of variants of a polynucleotide encoding a plurality of genes under conditions whereby one segment serves as a template for extension of another segment to generate a population of recombinant polynucleotides. This population is screened for a recombinant polynucleotide encoding a plurality of genes having a desired property.

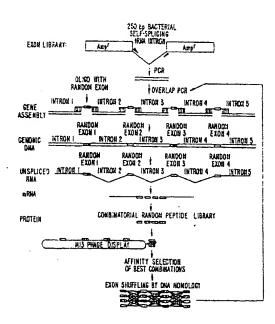


FIG. 20.

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EUROPEAN SEARCH REPORT

Application Number EP 98 12 2014

Category	Citation of document with indica of relevant passages		Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.CI.6)	
Y	WO 95 22625 A (AFFYMAX WILLEM P C (US); CRAME 24 August 1995 * the whole document *	TECH NV ;STEMMER RI ANDREAS (US))	1-20	C12N15/10 C12N15/64 C1201/68 //C07K14/435, C07K14/545, C12N9/86, C12N9/38, C12N15/52	
Y	STEMMER W: "DNA shuff fragmentatio and reass recombination for mole PROCEEDINGS OF THE NAT SCIENCES OF USA, vol. 91, October 1994, XP002087463 * the whole document *	embly: In vitro cular evolution" IONAL ACADEMY OF	1-20		
Y	STEMMER W P C: "Rapid protein in vitro by DN. NATURE,		1-30		
	vol. 370, 4 August 1994 XP002082182 * the whole document ~	4, pages 389-391.		750,000	
Y	W.P.C. STEMMER: "Searce" NATURE BIOTECHNOLOGY, vol. 13, June 1995, pag XP002095510	ges 549-553.	1-20 <u> </u>	TECHNICAL FIELDS SEARCHED (Int.Cl.6) C12N C12O	
	* the whole document *	-			
Y	US 5 279 952 A (WU KUN * the whole document *	C) 18 January 1994	1-20		
Y	US 5 223 408 A (GOEDDE) 29 June 1993 * the whole document *	L DAVID V ET AL)	1-20		
		-/	:		
	The present search report has been	drawn up for all claims	•		
	Place of search	Date of completion of the war n		Examiner	
	THE HAGUE	4 March 1999	: Hori	nig, H	
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EUROPEAN SEARCH REPORT

Application Number EP 98 12 2014

Category	Citation of document with ind of relevant passar		Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Y	R M HORTON ET AL: " genes without the us enzymes: gene splici extension" GENE, vol. 77, 1989, pages * the whole document	e of restriction ng by overlap 61-68. XP002090392	1-20	
	HO S N ET AL: "SITE-	 -DIRECTED MUTAGENESIS USING THE POLYMERASE nuary 1989, pages	1-20	
A	WO 91 07506 A (US GOV * the whole document	=== ERNMENT) 30 May 1991 *	1-20	
	HEIM R ET AL: "WAVEL POSTTRANSLATIONAL AUT FLUORESCENT PROTEIN" PROCEEDINGS OF THE NA SCIENCES OF USA, vol. 91, 1 December 1 12501-12504, XP000574 * the whole document	TIONAL ACADEMY OF 994, pages 454	1-20	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
A. CRAMERI ET AL.: fluorescent protein using DNA shuffling NATURE GENETICS, vol. 14. March 1996 XP002095449 NATURE PUBLISHING C * the whole documen		pages 315-319, , NEW YORK, US	1-16	
	The present search report has been	en drawn up for all claims		
		Date of completion of the search 4 March 1999	Horr	Exampler Lig, H
X : parti Y : parti docu	ATEGORY OF CITED DOCUMENTS cularly relevant if taken alone cularly relevant if combined with another ment of the same category nological background	L : document citad for	cument, out publis le in the application or other reasons	

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COLUSION NAVER FORMS (OLDS) AND THE SIALI



EUROPEAN SEARCH REPORT

Application Number

EP 98 12 2014

Category	Citation of document with indic	ation, where appropriate,		Relevant	CLASSIFICATION OF THE	
Jalegoly	of relevant passage	es		to claim	APPLICATION (Int.Cl.6)	
L	WO 97 35966 A (MAXYGE JEREMY (US); STEMMER	N INC ;MINSHULL WILLEM P C (US))		1-16		
	2 October 1997 * examples I-IV. *					
T	CRAMERI A ET AL: "Mo an arsenate detoxific shuffling" NATURE BIOTECHNOLOGY. vol. 15, May 1997, pa XP002082183 * the whole document	ation pathway y ges 436–438.	n of DNA	1-16		
				:		
				7	TECHNICAL FIELDS SEARCHED (Int.Cl.6)	
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	The present search report has been	drawn up for all claims				
	Place of search	Date of completion of the		7	Examiner	
	THE HAGUE	4 March 1999	9	Horr	nig, H	
X : parti Y : parti	ATEGORY OF CITED DOCUMENTS cularly relevant if taken alone cularly relevant if combined with another ireact of the same category.	E ; earlier after the D : docum	patent docur is filing date ent cited in t	inderlying the in ment, but publis he application other reasons	nvention had on, or	
A : tech	document of the same category L : document A : technological background			the same patent family, corresponding		

EPO FORM 1503 03.02 (P04C01)

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ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO.

EP 98 12 2014

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on The European Patent Office is in no way liable for these particula is which are merely given for the purpose of information.

04-03-1999

	Patent documer ed in search rep		Publication date		Patent family member(s)	Publication gate
WO	9522625	A	24-08-1995	US AU CA CN EP JP US US	5605793 A 2971495 A 2182393 A 1145641 A 0752008 A 10500561 T 5811238 A 5837458 A	25-02-199 04-09-199 24-08-199 19-03-199 08-01-199 20-01-199 22-09-199 17-11-199
US	5279952	Α	18-01-1994	NONE		
US	5223408	Α	29-06-1993	US	5736135 A	07-04-199
WO	9107506	Α	30-05-1991	UA	6886991 A	13-06-199
WO	9735966	А	02-10-1997	US AU WO AU CA EP WO	5837458 A 2337797 A 2542697 A 9735957 A 1087397 A 2239099 A 0876509 A 9720078 A	17-11-199 17-10-199 17-10-199 02-10-199 19-06-199 05-06-199 11-11-199
						·

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